Project Title: **Effects of Oiled Incubation Substrate on Pink Salmon Reproduction**

Project Number:	00476
Restoration Category:	Research
Proposer:	Ron Heintz NMFS, Auke Bay Laboratory ABL Program Manager, Dr.Stan Rice NOAA Program Manager: Bruce Wright
Lead Trustee Agency:	NOAA
Cooperating Agencies:	ADF&G
Alaska SeaLife Center:	No
Duration:	Second of 3 years
Cost FY00:	$81,700
Cost FY01:	$36,000
Geographic Area:	Little Port Walter, Baranof Island, Southeast Alaska
Injured Resource:	Pink salmon

ABSTRACT

This project examines the effects of oil exposure during embryonic development on the gamete viability of pink salmon that survive to spawn. The objective is to determine if exposure to oil during incubation could explain the reduced gamete viability reported for pink salmon in Prince William Sound under Restoration Study 191A. In that study eggs taken from pink salmon returning to oiled streams had higher mortality rates than eggs taken from salmon in unoiled streams. These data suggest a dramatic effect of oil on vertebrate reproduction that has not previously been described. The plausibility of reduced gamete viability is suggested by effects demonstrated in 191B, including reduced marine survival and growth of returning adults; however this effect still requires unequivocal demonstration. This is the second year of this study. During the first year, fry were exposed, marked and released. During the second year, adults will be recovered and their gametes crossed to demonstrate their viability. Estimates of viability will be obtained in the third year of the project and these will be used to complete a model of life cycle effects resulting from incubation of eggs in oiled gravel.

INTRODUCTION

This experiment tests the hypothesis that incubation in water contaminated by oiled gravel produces adult pink salmon with reduced reproductive capacity. After the Exxon Valdez oil spill (EVOS), pink salmon embryos developing in oiled streams experienced increased mortality (Bue et al. 1995). Further experiments reported by Bue et al. (1998) indicated that adult fish returning to oil contaminated streams had reduced gamete viability. In these experiments, gametes were collected from adults returning to oil contaminated and uncontaminated streams and incubated in a hatchery before they could be they could be exposed to oil. Despite the identical incubating environments, the gametes derived from oil contaminated streams consistently produced fewer viable embryos than gametes derived from uncontaminated streams. This difference was thought to result from differences in the incubating environments experienced by the adults contributing the gametes and therefore suggested a previously undescribed long-term effect of oil on reproductive ability.

Demonstrating a long-term effect of oil on pink salmon reproduction has important implications for managers in Princce William Sound as well as managers seeking to restore wildlife populations in other locations. The effects observed in pink salmon after the EVOS were a direct result of their dependence on the intertidal environment for early development. This implies that other species with similar dependencies were also at risk. Furthermore, the exposure levels shown capable of causing long-term impacts on growth and marine survival in pink salmon are less than or equal to the Alaska State water quality criteria, which are among the most rigorous in the United States. This suggests that water quality criteria in locations outside Alaska may limit the potential for restoring fish populations in streams located near hydrocarbon sources such as urban runoff.

The intent of this experiment is to examine the effects of oil exposure on pink salmon reproduction under controlled laboratory conditions. Environmental exposures will be simulated by incubating embryos in gravel with a known concentration of oil from fertilization to emergence in a simulated intertidal environment. The fish will be marked and released. Upon maturity, returning adults will be recovered and the viability of their gametes will be compared to those taken from unexposed, but similarly handled, fish.

The procedure proposed here repeats the experiments performed under Restoration 191B, but with the sole purpose of testing the hypothesis that incubating in oiled gravel impairs the reproductive ability of salmon that survive to maturity. Consequently, we have limited the exposures to two doses, released sufficient numbers of fish to guarantee an adequate number of returning adults, and marked the fish externally so that exposure levels can be readily discerned when the fish return to spawn. These procedures significantly reduce the cost of the study.

Projects 191B and 076 were successful in measuring oil impacts to marine survival and straying,

but the coded-wire tags required to identify the treatment groups in that study had to be recovered and decoded before adult pairs could be matched for mating. The delays encountered while decoding hundreds of tags led to reduced gamete viability in all the treatments, which may have masked any oil related effects. This follow-up study uses external marks to alleviate that problem and is designed specifically to test for oil effects on gamete viability.

NEED FOR THE PROJECT

A. Statement of the Problem

Field and laboratory work conducted after the EVOS by Restoration Study 191A suggested that pink salmon in contaminated streams had reduced fitness when they were exposed to low concentrations of polynuclear aromatic hydrocarbons (PAH). Field evidence for reduced fitness included observations of increased embryo mortality in contaminated streams (Bue et al. 1995) and reduced viability in gametes taken from adults returning to contaminated streams (Bue et al. In press). These data have been supported by laboratory studies (Heintz et al. 1995 and Wertheimer et al. 1996) that have shown the sensitivity of pink salmon embryos to water contaminated with very low concentrations of oil.

The laboratory studies provided a basis for estimating the total reduction in fitness for pink salmon exposed to water contaminated with oil at concentrations approaching those prescribed by the Alaska State water quality criteria. The reductions in embryo survival, growth, and marine survival can be integrated to calculate a total reduction in the average fitness for exposed populations of nearly 50%. However, reduced gamete viability among individuals as reported by Bue et al. (In Press) has not been adequately demonstrated among the survivors of the laboratory exposures. In 1995, gametes taken from fish exposed as embryos in the 1993 experiments appeared to have reduced viability, but inadequate numbers of fish prevented statistical verification of this observation. In 1997 we recovered sufficient numbers of fish that had been exposed as embryos in 1995 experiments, however high mortality rates were observed in all the treatment groups including the controls, possibly masking elevated mortality rates in the exposed groups. The source of these high mortality rates is unknown, but is probably related to the time required to hold the gametes prior to spawning in order to find sufficient numbers of mates among all the returning fish.

The effects already described for pink salmon that incubate in oil gravel suggest the plausibility of reduced gamete viability. These include effects on fitness related characters such as growth and marine survival. In addition, histopathological examination of fry emerging from oiled gravel demonstrated an effect of oil on gonad development (Marty et al. 1997). Previous attempts to demonstrate gamete viability have provided results that are highly suggestive of oil related effects, and have generally included exposure to a number of doses to allow generation of dose response curves. In the study proposed here, the design is aimed at demonstrating an effect of oil exposure on gamete viability. Thus, we have limited the number of treatments in order to maximize the number of fish that survive to adult, and we will mark fish externally to identify exposure level to minimize the holding time for gametes prior to fertilization.

B. Rationale/Link to Restoration

Pink salmon are an ideal species for identifying prolonged population effects resulting from embryonic oil exposure. Pink salmon have been widely studied because of their commercial value and relatively simple life history, and their dependence on the intertidal for incubating in PWS made them a premier sentinel species for detecting oil damage after EVOS. Consequently, a large amount of effort and money was expended towards understanding how oil affected pink salmon populations. This work has led to important advances in our understanding of the scope and mechanisms of oil toxicity and has led to developing a model describing the average reduction in reproductive fitness of exposed populations. Laboratory confirmation of Bue et al.'s claim of an oil effect on gamete viability for pink salmon is the last piece of data required to construct a new paradigm for oil toxicity .

Confirmation of the field observations of reduced gamete viability (Bue et al. 1995) will provide managers with a more comprehensive model for the long-term effects of oil on pink salmon. This information is important to managers working to restore salmon populations in PWS as well as locations in less pristine locations. Concentrations found to be effective at reducing average fitness (Heintz 1995) are significantly lower than those required by the Alaska State water quality criteria and are typical of concentrations in urban locations (Maltby et al. 1995). Of additional value is the demonstration that oil has life long effects for organisms exposed during embyonic development. Both the exposure mechanism and the extent of the effects described in this work represent significant advances in the understanding of oil toxicity.

C. Location

This project is underway at Little Port Walter (LPW), a research hatchery operated by NMFS in southeastern Alaska. This location is appropriate because it has been the site of these studies since their inception. The facility provides easy access to the intertidally spawning pink salmon stock that has been the subject of previous experiments. In addition, the exposure apparatus requires a simulated intertidal environment and such a system is in operation at LPW.

COMMUNITY INVOLVEMENT AND TRADITIONAL ECOLOGICAL KNOWLEDGE

This project will take place in southeastern Alaska, but depends on contract labor for marking fish for a period of 6 weeks in the spring of 2000. All efforts will be made to advertise our labor requirements in the spill zone. We will continue to provide information to interested public (primarily fishermen) who visit the station; we will be displaying at the facility the posters developed for the Restoration Workshop for 97191B and 97076 as interpretative tools. In addition, in 1999 we have traveled to Cordova to present a summary of our results to the public.

PROJECT DESIGN

A. Objectives

1. Determine the effect of incubating in oiled gravel on reproductive capacity of pink salmon.

2. Complete the model of life cycle impacts from incubation in oiled gravel.

We are currently testing the hypothesis that incubating in gravel contaminated with oil leads to reduced gamete viability. Fish have been exposed, marked and released. Gametes will be collected at the end of FY 00. Examination of gamete viability will provide information for completing a life-history model for oil toxicity which allows us to quantify the effect of oil on each of the major life-history stages of pink salmon in terms of reduced survival. Thus far, we have demonstrated that embryos developing in oil contaminated gravel have reduced survival, and fry that survive incubation have reduced growth and reduced survival to maturity. These observations account for a 50% reduction in the average survival of a population of pink salmon exposed to PAH concentrations equal to the Alaska State water quality standard. The proposed study will refine the model by providing an estimate of the specific loss in reproductive ability in exposed individuals. To our knowledge this type of analysis does not exist for any vertebrate and these effects occur at concentrations that are commonly seen in urban locations.

B. Methods

The exposure mechanism and fish culture procedures followed those described in previous proposals for Restoration Study 191B. Gametes were taken from an intertidally spawning pink salmon stock, transferred to our hatchery at Little Port Walter where they were incubated beginning in FY98. The eggs were exposed to effluent from either oil-coated or untreated gravel. In FY99, approximately 60,000 surviving fry from each exposure group were marked by excising the adipose fin and one pelvic fin depending on exposure regime. Marked fish were held for a short period to recover from the marking procedure and then released. Exposures began in September of 1998; between 50 and 500 mature fish representing each treatment are expected to return in September 2000.

All pink salmon returning to the Sashin Creek weir will be inspected for marks during the 2000 escapement period (FY00). Marked fish will be sorted by treatment groups into holding pens since the external marks will provide for immediate identification of exposure level. On a given spawning date, sufficient numbers of fish will be removed from each pen and spawned, ensuring minimal holding times for gametes prior to spawning. Spawning will be directed by a contracted expert in fish reproduction to ensure maximal survival. Previously, we have released fish from multiple treatments which necessitated the use of coded-wire tags for identifying them upon return. This approach allowed us to quantify oil effects on growth, marine survival, and homing fidelity but not gamete viability due to the long time periods associated with tag recovery decoding on a given spawning date.

Gamete viability will be determined for the oil treatment and the control groups. Three experiments will be performed to evaluate the reproductive viability of the parents. The objective of the first experiment will be to determine the average offspring survival of parents exposed to oil during incubation . The importance of this experiment is that all the possible crosses within an exposure group can be made and the overall average survival measured, however the primary source of variation will be measurement error and no information will be available on individual variation. Therefore, the second experiment's objective will be to estimate how much of the variability in offspring survival is due to individual variation. This experiment will determine individual variability and thus provide control for the interpretation of the results of the first experiment. Lastly, the objective of the third experiment is to identify the genetic component to variability or heritability of offspring survival. The benefit of this third experiment, besides demonstrating a genetic effect of oil, is that calculation of the genetic heritability of the damage provides a basis for calculating how long the effect will persist in the exposed population. In all experiments survival will be measured to fertilization, eyeing, and emergent fry stages. The numbers of defective or dead progeny will be compared between treatment groups. Because these gametes will not be incubated in an oiled environment, any observed increases in mortality or defective individuals can be attributed to oiling effects upon the first generation.

First Experiment

Average offspring survival will be estimated in the first experiment by measuring the survival in pools of gametes comprising all the possible pairwise crosses. On each day of spawning, 2 embryo pools will be formed per treatment. Upon formation of an embryo pool, 6 subsamples, each of approximately 150 embryos, will be randomly selected and incubated in an individual cell within a Heath tray. On a given day, pools will be formed by randomly assigning half the males and females from a treatment group to one of two subgroups. Each female in a subgroup will contribute approximately 900 eggs to a common pool, the pool will be mixed and the mixture divided into a number of aliquots equal to the number of males in the subgroup. Each male in the subgroup will fertilize one aliquot, and the fertilized eggs will be recombined in a common container, mixed and divided into six aliquots that will be incubated in randomly assigned locations. Thus, the average survival of a treatment group on a given day will be the mean of the average survivals in each of the two subgroups.

The estimates of mean survival of the treatment groups will be compare with *t* tests after assuming that variability between groups of like-treated incubators is negligible. A *t* test between, for example, treatment 1 and 2, when there are *d* spawning days, *q* treatments, *p* subgroups per treatment, and *r* cells per subgroup will have the following form:

$$t_{((p-1)*q*d)df} = \frac{\frac{1}{d}[\overline{sv_{11}} + \ldots \overline{sv_{1d}} - \overline{sv_{21}} \ldots - \overline{sv_{2d}}]}{\sqrt{\frac{1}{d^2} * \frac{s_c^2}{p*r} *2*d}}$$

where,

$\overline{sv_{ij}}$ = Survival rate for treatment *I* on day *j*

s^2_c = Combined Between-Pools Mean Square obtained by ANOVA.

Comparisons will be made between each of the doses and the control and the overall a will be maintained at 0.05.

Second Experiment

For the second experiment, fish from the oil and control doses will be mated using a fully-crossed half-sib design (Falconer 1981). In this design, the remaining eggs from an exposed female and a control female are each split into two aliquots. One aliquot from each female is fertilized with aliquots of sperm from the same oil-exposed male, and one aliquot from each female is fertilized with aliquots of sperm from the same control male. This 2 x 2 breeding matrix will be replicated so that every female is represented in a breeding matrix or until there are 30 breeding matrices for each treatment, whichever is greater. Each half-sib family will be incubated in an individual container.

Third Experiment

The third experiment will be performed under contract by the University of Alaska using gametes collected at the same time as those used in the previous experiments. The fish will be used to produce ten 2 x 3 mating sets: 'oiled' females crossed with oiled males and ten 2 x 3 mating sets: 'unoiled' females crossed with unoiled males. Within each set, eggs from each female will be separately fertilized using semen from 3 males. Therefore, each set will produce 6 families, resulting in a total of 60 oiled families and 60 unoiled families (oiled and unoiled F1). Each family will be divided in 2 parts, each of which will be randomly placed in an incubator compartment. Data to be collected for each of the 240 incubator compartments includes: mortality rate at eye, hatch, and emergence, and developmental rate to eye, hatch, and emergence.

Additive genetic, maternal, non-additive genetic, and phenotypic variances will be estimated and heritabilities, and ratios of maternal and nonadditive genetic variances to phenotypic variances will be calculated using an animal model solved by applying a derivative free technique for estimating variance components employing restricted maximum likelihood (Graser et al., 1987). The

derivative-free restricted maximum likelihood (DFREML) analysis procedure of Meyer (1988) will be utilized. The technique has been utilized to analyze data from breeding experiments of fish (Crandell and Gall, 1993). Heritability estimates may be used to predict expected genetic change due to natural selection for a range of selection intensities (Van Vleck, 1987).

C. Cooperating Agencies, Contracts and Other Agency Assistance

Fish spawning and handling of gametes in FY 00 will be directed by a contracted expert in the field of fish reproduction. The statistical analysis of the results for experiment 1 have been designed by the Alaska Department of Fish and Game (ADF&G). The design and execution of experiment 3 will be contracted to University of Alaska through ADF&G.

SCHEDULE

A. Measurable Tasks for FY 00 (October 1, 1999 - September 30, 2000)

Sept. 2000: Collect gametes from returning adults and cross them.

B. Project Milestones

Completed:
Sept. 1998: Set-up exposure apparatus, collect gametes, begin exposures.

May 1999: Release marked fry

Underway:
Sept. 2000: Examine oil effect on gamete viability by recovering and spawning marked adults when they return to weir.

Aug 2001: Complete analysis of gamete viability.

C Completion Date

Final Report will be submitted on August 15, 2001 in FY 2001.

PUBLICATIONS AND REPORTS

FY 99: Annual Report describing the doses, exposure apparatus and effects on early incubation.

FY 00: Final Report

Other manuscripts planned:

Heintz, R. 2000. Effect of incubating in oil on pink salmon reproductive capacity. Journal Unknown.

Heintz, R. 2000. Incubating in oiled gravel damages the entire life-history of pink salmon. Journal Unknown.

PROFESSIONAL CONFERENCES

No conferences planned in FY 00, travel to 2000 Oil Spill Symposium is covered in other Proposed Project Plans.

NORMAL AGENCY MANAGEMENT

This project will complete the work begun under Restoration 191B which has been performed cooperatively between the Trustees and NMFS from the outset. However, NMFS proposes providing most labor requirements for this project and seeks funding for primarily contractual labor and commodities. There is no charge for project support costs which include management of the LPW facility and project budget, production of reports or hydrocarbon chemistry to verify dosing. There was no charge for setting up the experiment in FY98 and early FY99, NMFS covered costs associated with setting up the exposure apparatus, spawning pink salmon, and maintaining the incubation for 9 months. In outlying years, NMFS will cover costs associated with the several man-weeks associated with spawning the returning fish, and evaluating their gamete viability.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

This project will be coordinated with continuation of ADF&G research and monitoring efforts regarding pink salmon embryo survival under Restoration 191A. This study also coordinates the results of Restoration 191B and 076 by completing a life-history model for oil effects on pink salmon. Investigators and agencies will coordinate by sharing data. NOAA/NMFS will coordinate with the Trustees by providing labor requirements and laboratory overhead.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR

Ron Heintz has been involved in examining the effects of *Exxon Valdez* oil on pink salmon since 1992. He has developed the methods proposed for this project, published 3 papers has another in review on this topic. In addition, he has presented results of these studies at 10 professional meetings.

OTHER KEY PERSONNEL

Robert Bradshaw will assist in all the fish culture and logistics.
Stan Rice and Jeff Short will assist in data interpretation.

LITERATURE CITED

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